Sensitizing Cancer Cells: Is It Really All about U?

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http://dx.doi.org/10.1016/j.ccr.2012.06.010

In this issue of *Cancer Cell*, Hu et al. report that TMPK and RNR, two key enzymes in deoxyribonucleotide biosynthesis, co-localize to damaged DNA and produce nucleotides necessary for DNA repair while suppressing uracil incorporation. TMPK inhibition disrupts this balance and selectively sensitizes cancer cells to low-dose chemotherapy.

An ample and properly constituted supply of deoxyribonucleotides is required for the successful completion of DNA replication and repair. Consequently, the cellular enzymes responsible for nucleotide biosynthesis have long been recognized as possible targets for anti-cancer drugs; one such therapeutic is 5-fluorouracil (5-FU). 5-FU is converted intracellularly into toxic metabolites that are incorporated into nucleic acids and additionally inhibit thymidylate synthase (TS), a crucial enzyme for de novo dTTP biosynthesis (Figure 1) (Longley et al., 2003). 5-FU has been used in the clinic for over 50 years and is still being used for the treatment of colorectal and other cancers. However, 5-FU treatment has toxic side-effects, and its use is further limited by the occurrence of resistance. In this issue of *Cancer Cell*, Hu et al. (2012) describe exciting findings that identify thymidylate kinase (TMPK), another key player in thymidine nucleotide biosynthesis, as a promising target among the nucleotide biosynthetic machinery by virtue of its newly discovered role in generating dTDP for DNA repair directly at sites of DNA damage.

Of the four classical deoxyribonucleotides, thymidine is distinguished by both its metabolic regulation and the way it is utilized. Thymidine is the only nonessential deoxyribonucleotide for DNA synthesis, as DNA polymerases typically fail to distinguish between dTTP and dUTP during DNA replication and repair and can incorporate dUTP into DNA when dTTP is limiting. Furthermore, while the synthesis of cytosine and purine nucleosides through de novo and salvage pathways occurs in the cytoplasm, the salvage and folate-dependent de novo synthesis of thymidylate, catalyzed by thymidine kinase and TS respectively, occurs in the nucleus at sites of DNA synthesis (Anderson et al., 2012; Chen et al., 2010). Cellular dTTP pools are maintained at very low levels, and both pool depletion and expansion affect DNA integrity and human health (Samsonoff et al., 1997). There is increasing evidence that dTTP is synthesized "on-site and on-demand", and loss of the capacity to synthesize dTTP results in dU accumulation in DNA, causing genomic instability through futile cycles of DNA synthesis and repair (Blount et al., 1997).

Previous reports indicated that targeting dTTP production through TMPK depletion sensitizes cells to double strand DNA breaks (DSB) but, importantly, does not appear to impair cell viability in the absence of exogenous genotoxins (Hu and Chang, 2008). The present study extends those intriguing observations with the finding that TMPK knockdown leads to increased and persistent DNA lesions following treatment of cells with the clastogen doxorubicin. These effects were associated with increased uracil content in DNA and could be countered by overexpression of dUTPase, the enzyme that removes dUTP from the nucleotide pool.

The production of dUTP requires ribonucleotide reductase (RNR), a heteromultimer composed of large (R1) and small (R2) subunits that reduces NDPs to form dNDPs. The subcellular localization of mammalian RNR proteins has been a matter of debate (Pontarin et al., 2008), although recent evidence suggests that at least some RNR complexes localize to sites of DNA damage in the nucleus, where they can contribute to nucleotide production for DNA repair (Niida et al., 2010). Interestingly, the faulty DSB repair following TMPK knockdown could be rescued by disrupting RNR recruitment to DNA lesions, and additional experimental manipulation of RNR expression established RNR levels as a critical determinant of DSB repair proficiency following TMPK impairment. The authors proceed to show that TMPK, like RNR, is present in the nucleus at sites of DNA damage. The convergence of these pathways where DNA repair is occurring provides new insights into mechanisms of dUTP synthesis and incorporation into DNA during DSB repair. TMPK is essential for dTDP synthesis from both the de novo and salvage thymidylate synthesis pathways, whereas RNR generates dUDP in the process of producing dNDPs needed for DNA replication and repair, raising the possibility of coordinated regulation of dTDP and dUDP levels in the nucleus at sites of DNA damage.

Taken together, the results point toward a model (Figure 1) in which the balanced activity of TMPK, RNR, and other factors creates a local environment with a low ratio of dUTP to dTTP, limiting dUTP incorporation during DNA repair under normal circumstances. When this regulatory network is perturbed by inhibition of TMPK, dTTP levels decrease, leading to increased uracil incorporation during the DNA synthesis step of homologous recombinational repair. This results in an unproductive and ultimately lethal cycle of events in which the incorporated uracils are targeted for excision from the DNA, and the region is resynthesized under the same unfavorable nucleotide pool conditions with a high dUTP to dTTP ratio.

The translation of these findings to cancer therapy seems promising but remains to be proven. Inhibition of TS by 5-FU treatment is cytotoxic to both normal

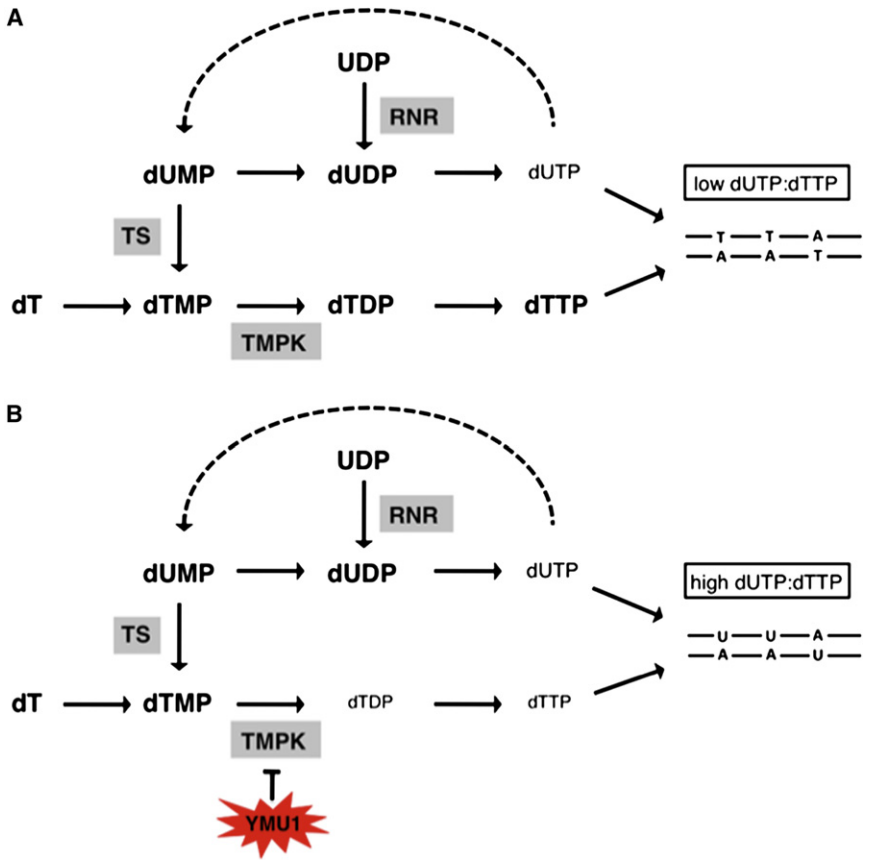

Figure 1. TMPK Co-Localizes with RNR at DNA Repair Sites to Limit Uracil Incorporation during DNA Synthesis

(A) Under normal conditions, a low dUTP:dTTP ratio is maintained in part through the breakdown of dUTP by dUTPase (dotted arrow) and TMPK-dependent dTTP biosynthesis.

(B) When TMPK is inhibited, such as by the small molecule YMU1, dTTP levels drop, resulting in a high dUTP:dTTP ratio that promotes uracil incorporation into DNA. Uracils in DNA are then targeted by uracil DNA glycosylase and excised, leading to futile repair cycles and DNA breakage (not shown).

RNR, ribonucleotide reductase; TMPK, thymidylate kinase; TS, thymidylate synthase.

and tumor cells, and its effects are associated with the incorporation of both dU and 5FdU into DNA. The authors propose that TMPK may be a more desirable target for specifically sensitizing tumor cells as compared to established therapeutic approaches directed against TS and the de novo thymidylate synthesis pathway without specificity for transformed cells (Longley et al., 2003). The authors propose that a cancer cell's Achilles heel is its elevated R2/TMPK ratio, leading to an increased dependence of cancer cells on TMPK activity to prevent uracil misincorporation in DNA. Moreover, R2 levels increase after DNA damage, such as that caused by doxorubicin and other chemotherapeutics, in part through a recently identified mechanism involving Cyclin F (D'Angiolella et al., 2012), potentially further tilting the dUTP:dTTP ratio at DNA damage sites in an unfavorable direction when TMPK is inhibited. These effects may be compounded in cancer cells because they often have cell cycle checkpoint defects and an increased S-phase fraction, which could contribute to high RNR activity as well as more opportunities for usage of homologous recombination for DNA repair.

The development of their lead compound, YMU1, offers some hope that this strategy could be realized. This selective, cell permeable TMPK inhibitor, identified by screening a small molecule library, has an IC_{50} of 0.6 μM. Similar to the effect of TMPK knockdown, treatment of cells with YMU1 alone does not significantly affect cell proliferation or cause cytotoxicity. It remains unclear why cells can tolerate TMPK inhibition in the absence of extrinsic stresses given the integral role of TMPK in dTDP production. The authors speculate that cells may produce variant isoforms of TMPK that could account for residual dTDP production after TMPK inhibition. Clearly, additional genetic and biochemical analyses are called for as TMPK continues to be explored as a drug target. Nevertheless, YMU1 sensitizes cultured tumor cells to low dose doxorubicin treatment, resulting in increased DNA damage and enhanced cell killing. Initial studies in a tumor xenograft mouse model further suggest that YMU1 in combination with doxorubicin suppresses tumor growth in vivo. Even before TMPK inhibitors are further evaluated for potential use in patients, the findings of Hu et al. (2012) suggest that monitoring the relative protein levels of R2 and TMPK in cancers could be predictive of chemosensitivity. While it remains to be seen whether this will be the case, the new focus on TMPK raised by this work has the potential to extend the long history of using the knowledge of the fundamentals of nucleotide metabolism for therapeutic benefit.

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